Immunologic Effects of Dioxin: New Results from Seveso and Comparison with Other Studies

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Animal studies indicate that the immune system is one of the most sensitive targets of the toxic effects of 2,3,7,8-tetrachloro-*p*-dibenzodioxin (TCDD). TCDD inhibits immunoglobulin secretion and decreases resistance to bacterial, viral, and parasitic infections in exposed animals. Nearly 20 years after the Seveso, Italy, accident, we measured immunoglobulin and complement plasma levels in a random sample of the population in the most highly exposed zones (n = 62) and in the surrounding noncontaminated area (n = 58). Plasma IgG levels decreased with increasing TCDD plasma concentration (r = –0.35, p = 0.0002). Median IgG concentration decreased from 1,526 mg/dL in the group with the lowest (< 3.5 ppt) TCDD levels to 1,163 mg/dL in the group with the highest (20.1–89.9 ppt) TCDD levels (p = 0.002). The association was significant (p = 0.0004) after adjusting for age, sex, smoking, and consumption of domestic livestock and poultry in multiple regression analysis and persisted after exclusion of subjects with inflammatory diseases and those using antibiotics or nonsteroidal anti-inflammatory drugs. IgM, IgA, C3, and C4 plasma concentrations did not exhibit any consistent association with TCDD levels. We performed a systematic review of all the articles published between 1966 and 2001 on human subjects exposed to TCDD reporting information on circulating levels of immunoglobulins and/or complement components. The literature indicates that the evidence for effects of TCDD on humoral immunity is sparse. Methodologic issues, results, and possible sources of variation between studies are discussed. The possible long-term immunologic effects of TCDD exhibited by the participants of the present study, coupled with the increased incidence of lymphatic tumors in the area of the accident, warrant further investigation. *Key words*: complement, dioxin, environmental disasters, immune system, immunoglobulins, tetrachlorodibenzodioxin. *Environ Health Perspect* 110:1169–1173 (2002). [Online 27 September 2002]
http://ehpnet1.niehs.nih.gov/docs/2002/110p1169-1173baccarelli/abstract.html

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCCD) has a long biological half-life in humans (> 7 years) and induces carcinogenicity and severe developmental, endocrinologic, immunologic, and reproductive toxicity in animal experiments (Birnbaum and Tuomisto 2000; Dragan and Schrenk 2000; IARC 1997). The immune system is one of its most sensitive targets (Birnbaum and Tuomisto 2000). TCDD inhibits immunoglobulin secretion and decreases resistance to bacterial, viral, and parasitic infections in exposed animals (Birnbaum and Tuomisto 2000; Holsapple et al. 1991; Nohara et al. 2002). In humans, increased incidence of and mortality from lymphatic tumors have been found in exposed subjects, even though there is a lack of consistency among studies (Bertazzi et al. 2001; IARC 1997). Previous investigations have shown alterations in humoral immunity, but with no consistent dose–response relationship and often with poor control for confounders (Neubert et al. 2000).

In 1976 an industrial accident contaminated a residential area surrounding Seveso, Italy, with high levels of TCDD. The exposure was acute, relatively pure, and affected more than 45,000 men, women, and children. Measurements of soil levels of TCDD within 5 weeks delimited three zones (A, B, and R) of decreasing contamination (Bertazzi and Di Domenico 1994). Twenty years after the exposure, TCDD levels were still high in exposed persons, particularly in women. Plasma TCDD ranged between 9.8 and 89.9 ppt in individuals from zone A, 1.0–62.6 ppt in individuals from zone B, and 1.0–18.1 ppt in individuals from the noncontaminated surrounding area (non-ABR) (Landi et al. 1997, 1998).

In the present article we report on plasma immunologic parameters measured about 20 years after the accident in a random sample of the population of the area. Previous human studies investigating the effects of TCDD on humoral immunity are also discussed.

Materials and Methods

Study subjects and laboratory methods. The selection of subjects, their recruitment, and their characteristics, as well as dioxin assay methods, have been described elsewhere (Landi et al. 1997, 1998). Briefly, we randomly sampled 62 subjects from the most highly contaminated zones (A and B) and 59 subjects from the surrounding noncontaminated area (non-ABR zone). Study subjects were frequency matched by sex, decade of age, and cigarette smoking status. Residence in the specific zone was verified at recruitment. We obtained approvals of the institutional review boards and written informed consent from each study subject. A structured questionnaire, which included the collection of a detailed personal medical history and medication use in the week before the study, was administered to all subjects through personal interview.

We determined internal dose of TCDD using high-resolution mass spectrometric analysis performed on human plasma, as described by Patterson et al. (1987). Eleven subjects (4 from zone B and 7 from zone non-ABR) were excluded from the analyses based on blood TCDD because of missing data. Standard clinical hematologic and chemistry assays were performed. We quantified plasma immunoglobulins IgA, IgG, and IgM, and complement components C3 and C4 using common nephelometric methods (Thomas 1990). We used plasma protein electrophoresis to exclude monoclonal immunoglobulins. One subject from zone non-ABR was excluded from the study because of missing immunologic evaluation. We performed all assays blinded to subject's exposure status.

Statistical methods. We used nonparametric tests (Wilcoxon rank-sum, Kruskal-Wallis) for group comparisons. We performed simple and multiple regression analyses to assess correlations between variables. Several variables, such as age, sex, smoking status, body mass index, consumption of domestic livestock and poultry, alcohol consumption, acute and

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We are grateful to the study participants and to A. Carobene and P.A. Bertazzi for their insightful comments.

This work was supported by the government of the Lombardy Region, with additional funding from the Italian Ministry of University and Scientific Research, and the Foundation "Lombardia per l'Ambiente."

Received 8 March 2002; accepted 8 May 2002.

chronic medical conditions, and current medication use were possible confounding factors. The variables adjusted for in multiple regression models were selected on the basis of their correlation with TCDD and the immunologic parameters of interest in univariate analysis. All analyses were performed using the Stata statistical package, Release 7.0 (Stata Corporation, College Station, TX, USA).

Literature search. To document any published association between TCDD and immunologic parameters, in February 2002 we performed a PubMed (National Library of Medicine, Bethesda, MD, USA) search of all studies published from 1966 through December 2001. The main features of the studies and results are summarized in Table 1. We used the search query ["immune system," "immunity," "allergy and immunology," "immunocompetence," "immunologic," "immunoglobulins," "gamma globulins," "antibody formation," "IgA," "IgM," "IgG," "complement," "C3," OR "C4,"] AND ["TCDD," "tetrachlorodibenzodioxin," OR "dioxins"]. All citations and online abstracts were examined. We reviewed all the studies on human subjects exposed to TCDD reporting information on circulating levels of IgG, IgM, IgA, and/or complement components. Studies on subjects exposed to organochlorine compounds that did not include TCDD were not considered. Details on number of exposed and nonexposed (control) subjects, sex and mean

Table 1. Exposure to 2,3,7,8-tetraclorodibenzo-*p*-dioxin and levels of immunoglobulins and complement components in human subjects. Main features and results of the studies published between 1966 and December 2001.

Study		Subjects				Exposure					Results					
Reference	Setting	No. exposed	No. controls	Sex	Mean age (years)	Toxicants	Acute vs. chronic	Years from first exposure to the study	Assessment	TCDD internal dose	IgG	IgM	IgA	C3	C4	Control for confounders[a]
Pocchiari et al. 1979	Seveso, Italy	45	45[b]	M + F	3–7[c]	TCDD	Acute	≤ 1.5	Residence, chloracne	N/A	→	→	→	N/A[d]	N/A[d]	No
Hoffman et al. 1986	Quail Run Park, Missouri, USA	129	139	M + F	27.5	TCDD	Chronic	1–14	Residence history	N/A	→	N/A	N/A	N/A N/A	N/A N/A	Age, sex, socio-economic level
Jennings et al. 1988	Coalite Ltd, UK	18	15	M + F	49.8	TCDD	Acute	17	Presence at the accident/ clean-up	N/A	→	→	→	N/A	N/A	Age, sex, body weight, social class, alcohol, smoking
Webb et al. 1989	Missouri, USA	40	—	M + F	45.1	TCDD	Chronic	NR	Adipose tissue TCDD	ND–750 ppt	↑	→	→	N/A	N/A	Age, sex
Jansing and Korff 1994	Trichlorophenol plant, Germany	8	—	NR	49.6	TCDD	Chronic	15–28	Blood TCDD	163–1,935 ppt	γ-Globulins[e]			N/A	N/A	No
Ott et al. 1994	BASF AG, Germany	132 132	196/42[f] —	M M	60.7[g] 60.7	TCDD TCDD	Acute Acute	20–39 20–39	Work history Blood TCDD	1–553 ppt[g] 1–553 ppt	→ ↑	→ →	→ ↑	→ →	→ ↑	Sex Age, sex, body mass index, smoking
Marth et al. 1995	Garbage dumps, Egypt	100	98	M	10	PCDDs/PCDFs, PAHs, heavy metals	Chronic	NR	Residence	NR	→	N/A	→	N/A	N/A	Age, sex
Løvik et al. 1996	Frierfjord, Norway	24	—	M	46[h]	PCDDs/PCDFs	Chronic	NR	Blood PCDDs/PCDFs	NR	N/A	↓[i]	N/A	N/A	N/A	Sex
Halperin et al. 1998	NIOSH study subgroup	259	243	M	55.7	TCDD	Chronic	15–37	Work history[j]	ND–3,389 ppt[k]	↓[i]	→	→	↑[i]	N/A	Age, race, sex
Jung et al. 1998	Pesticide factory, Germany	187	—	Mainly M	56.0[h]	PCDDs/PCDFs	Chronic	> 10	Blood, serum, or adipose tissue TCDD	1.2–893 ppt	→	→	→	N/A	N/A	Age, smoking
Michalek et al. 1999	U.S. Vietnam Veterans	894	1,167	M	53.8	TCDD	Chronic	16–30	Blood TCDD	0–618 ppt	→	→	→	N/A	N/A	Age, sex, % body fat, race, military occupation, alcohol, smoking
Neubert et al. 2000	Boehringer, Germany (trial I)	88	—	Mainly M	36.5	PCDDs/PCDFs	Acute	NR	Blood PCDDs/PCDFs	1–140 ppt	↓[l]	→	→	N/A	N/A	No
	Rastatt, Germany (trial II)	70	—	Mainly M	51.5	PCDFs/PCDDs, lead, cadmium	Chronic	> 10	Blood PCDDs/PCDFs	2–17 ppt	→	→	→	N/A	N/A	No
	Trial I + trial II	158	—	Mainly M	43.1	PCDDs/PCDFs, lead cadmium	Acute + chronic	NR for trial I	Blood PCDDs/PCDFs	1–140 ppt	→[m]	→	→	N/A	N/A	Age, smoking, ESR, lymphoocytes/μL
Nagayama et al. 2001	Yusho poisoning	69	—	M + F	63.4	PCDFs, PCBs, PCDDs	Acute	29	Blood PCDDs/PCDFs,PCBs	NR[n]	→	→	→	N/A	N/A	No

Abbreviations: ESR, erythrocyte sedimentation rate; F, female; M, male; N/A, not available; ND, nondetectable; NR, not reported; PAHs, polycyclic chlorinated hydrocarbons; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; ↑, positive association; ↓, negative association; →, no significant association with the exposure.

[a]Most studies were restricted to males or to a specific age or were based on a matched design between exposed and nonexposed subjects; few studies used multivariable statistical models. [b]Number of controls not specified in the article, which summarized the results of periodical assessments of immunologic parameters; according to a later report by Sirchia (1982), control groups varied at each assessment (average sample size = 45). [c]Age range. [d]In the same study, an increase in total hemolytic complement activity (CH50) was reported by Sirchia (1982). [e]Negative association between total γ-globulin concentrations and TCDD blood levels (Spearman rank-correlation: $r_s = -0.71$, $p = 0.047$, calculated from data reported in the paper). [f]Immunoglobulin data from a convenience sample of 196 subjects were provided for comparison; a different referent sample ($n = 42$) was used for C3 and C4 results. [g]Mean age and TCDD levels of exposed subjects; blood TCDD was not assayed in controls. [h]Median age. [i]Results not shown, but only summarized in the paper; statistical significance was not reported. [j]Immune parameters not consistently related to TCDD serum levels after adjusting for age, alcohol, and smoking. [k]Serum TCDD measured in exposed workers and in 79 control subjects (< 20 ppt in controls). [l]Negative association with IgG and IgG1 levels. [m]Only results on IgG1 reported. [n]Study subjects were divided in three exposure groups according to the following TCDD toxic equivalent levels: < 70 ppt, 70–200 ppt, ≥ 200 ppt.

age of study subjects, characteristics of the exposure, time from the exposure, final results, and control for confounders were retrieved for each study. When the mean age of all study subjects was not reported, we computed it using individual age data, if available. Most articles specified the year of the beginning (YBE) and of the end (YEE) of the exposure, and the year of the beginning (YBR) and of the end (YER) of subjects' recruitment. If the time range from the first exposure to the enrollment in the study was not specified in the article, we derived it as lower limit = YBR – YEE; upper limit = YER – YBE. When multiple methods of exposure assessment were featured in a single study, we reported only that used in the statistical analysis of immunologic parameters. Study design and statistical methods were reviewed, and the methods for confounding prevention or control, such as matching, restriction, and multivariable regression analysis (Rothman and Greenland 1998) were ascertained. Immunologic parameters were considered associated with TCDD exposure when statistically significant results ($p < 0.05$) were reported.

Results

We found a significant decrease in IgG levels (Figure 1) in the exposed subjects. Plasma IgG ranged from 2,252 to 809 mg/dL and progressively decreased with increasing lipid-adjusted TCDD plasma concentration ($r = –0.35$, $p = 0.0002$). The finding was slightly more pronounced in female ($n = 53$, $r = –0.39$, $p = 0.003$) than in male subjects ($n = 56$, $r = –0.33$, $p = 0.02$). When individuals were divided in categories of internal dose according to TCDD quintiles (Table 2), median IgG concentration decreased from 1,526 mg/dL in the group with the lowest (<3.5 ppt) TCDD levels to 1,163 mg/dL in the group with the highest (20.1–89.9 ppt) TCDD levels (test for difference between groups, $p = 0.002$).

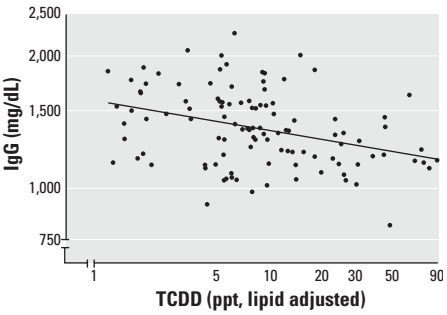

Figure 1. IgG plasma concentrations and plasma levels of TCDD in a random population sample of the highly contaminated zones (A + B) and of the surrounding noncontaminated area (non-ABR zone) of Seveso, Italy. Both variables are presented on a log-scale. $r = –0.35$; $p = 0.0002$.

Consistently, subjects from the most contaminated zones had lower IgG than subjects from the surrounding noncontaminated area. Median IgG levels were 1,403 mg/dL in the noncontaminated area (non-ABR, $n = 58$), 1,294 mg/dL in zone B ($n = 55$; $p = 0.03$ vs. non-ABR), and 1,142 mg/dL in zone A, the most contaminated area ($n = 7$; $p = 0.01$ vs. non-ABR).

We considered several factors that may have biased the results. We previously showed that in this population smoking and consumption of domestic livestock and poultry were among the major determinants of high TCDD plasma levels, together with female gender and older age (Landi et al. 1998). In the present study, median IgG levels were lower in current (1,202 mg/dL, $n = 22$) and former (1,281 mg/dL, $n = 28$) smokers, as compared with nonsmokers (1,424 mg/dL, $n = 59$; $p = 0.03$, test for difference between groups). Subjects who reported consumption of domestic livestock at the time of the accident ($n = 34$) had lower levels of IgG than those who did not report such consumption (1,228 vs. 1,417 mg/dL, $p = 0.06$). We used multivariable regression models to control for these possible confounding factors. After adjusting for age, sex, smoking, and consumption of domestic livestock, the inverse association between plasma TCDD and IgG remained highly significant ($p = 0.0004$). The results did not change after exclusion of an outlier subject who exhibited the lowest IgG levels ($r = –0.32$, $p = 0.0008$ in simple regression analysis, and $p = 0.002$ after adjusting for age, sex, smoking, and consumption of domestic livestock). Based on clinical chemistry assays and plasma protein electrophoresis, no subjects had monoclonal gammopathy, and four had increased C-reactive protein. No subjects reported treatment with immunosuppressive drugs, and five subjects had used antibiotics in the week before the study. An additional 22 individuals had taken aspirin or other nonsteroidal anti-inflammatory agents, mostly for headache, osteoarthritis, or menstrual pain. We repeated all statistical analyses excluding each or all the groups (involving a total of 28 subjects) mentioned above and did not find any substantial difference in the results (data not shown).

IgM, IgA, and complement component C3 plasma concentrations were not significantly associated with TCDD levels (Table 2). Complement component C4 concentrations were higher in the highest TCDD group (Table 2) and a positive trend was found in regression analysis ($r = 0.21$; $p = 0.03$). This association was not significant after adjusting in multivariable regression analysis for age ($p = 0.29$), or for age, sex, smoking, and consumption of domestic livestock ($p = 0.11$).

Overall, we retrieved 13 articles that reported results on immunoglobulin and complement levels in TCDD-exposed subjects (Table 1). Twelve studies measured immunoglobulins of one or more immunoglobulin classes. One article reported total γ-globulin levels (Jansing and Korff 1994). Four studies described a decrease in immunoglobulin levels (Halperin et al. 1998; Jansing and Korff 1994; Løvik et al. 1996; Neubert et al. 2000), and two studies reported increased IgG (Ott et al. 1994; Webb et al. 1989). Complement levels were determined in three studies, and only one of them included both C3 and C4 (Ott et al. 1994). C4 levels, but not C3, were positively associated with blood TCDD in this study. Increased total hemolytic complement activity (CH50) was described in children examined immediately after the Seveso accident (Pocchiari et al. 1979; Sirchia 1982). Halperin et al. (1998) reported increased C3 concentrations in the exposed subjects but no dose–response relationship with serum TCDD.

Five of the studies reported in Table 1 included subjects exposed not only to TCDD but also to other polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and, in some instances, to polycyclic chlorinated hydrocarbons (PAHs) and heavy metals. In other studies carried out

Table 2. Subjects' plasma immunoglobulins and complement components by TCDD plasma levels in Seveso, Italy.

	Plasma TCDD[a]										
	1.2–3.5 ppt ($n = 21$)		3.6–6.0 ppt ($n = 21$)		6.1–9.3 ppt ($n = 23$)		9.4–20.0 ppt ($n = 22$)		20.1–89.9 ppt ($n = 22$)		
	Median	Interquartile range[b]	Median	Interquartile range[b]	Median	Interquartile range[b]	Median	Interquartile range[b]	Median	Interquartile range[b]	p-Value[c]
IgG (mg/dL)	1,526	1,388–1,725	1,422	1,121–1,566	1,363	1,294–1,575	1,302	1,168–1,538	1,163	1,122–1,316	0.002
IgM (mg/dL)	168	126–188	134	107–183	164	92–265	173	112–233	135	86–165	0.42
IgA (mg/dL)	230	191–308	213	157–300	269	201–325	223	193–310	301	167–354	0.66
C3 (mg/dL)	76	67–85	69	61–78	71	63–78	75	64–86	76	61–87	0.64
C4 (mg/dL)	25	20–31	28	21–32	26	22–33	26	20–33	33	26–38	0.04

[a]Plasma TCDD quintiles were used to define TCDD categories. [b]25th–75th percentiles. [c]Kruskal-Wallis test for differences between TCDD categories.

in occupational settings, the presence of exposure to other chemicals cannot be ruled out. The sample size was generally small, with the exception of two investigations, which included more than 500 subjects (Halperin et al. 1998; Michalek et al. 1999). Most studies were restricted to males or to a specific age or were based on a matched design between exposed and nonexposed subjects. These methods allow for the control of a limited number of confounding variables. Five studies used multivariable statistical models (Halperin et al. 1998; Jung et al. 1998; Michalek et al. 1999; Neubert et al. 2000; Ott et al. 1994), but not all the potential confounders were considered in most of them.

Discussion

Nearly 20 years after the Seveso accident, we found a suppression of IgG plasma levels in subjects exposed to TCDD.

Numerous experimental studies showed that TCDD may suppress both humoral and cell-mediated immune responses (Birnbaum and Tuomisto 2000; Kerkvliet 2002). Inhibition of immunoglobulin secretion has been demonstrated in multiple animal species (Birnbaum and Tuomisto 2000; Nohara et al. 2002; Vorderstrasse et al. 2001), as well as in human lymphocytes cultivated *in vitro* (Sulentic et al. 2000). Zober et al. (1994) found an increased incidence of infectious and parasitic diseases in a small retrospective cohort study of TCDD-exposed workers, but clinical diagnoses were ill-defined.

The literature indicates that evidence for TCDD effects on humoral immunity is sparse. We reviewed all the articles published between 1966 and 2001 on subjects exposed to TCDD reporting information on circulating levels of IgG, IgM, IgA, and/or complement components (Table 1). Similarly to our findings, Neubert et al. (2000) reported a decrease in IgG levels, mainly of the IgG_1 subclass, the most prevalent of human IgG molecules, in workers exposed to PCDDs and PCDFs in Germany (Table 1, trial 1). It is suggestive that the subjects enrolled in this study had blood TCDD levels similar to those observed in our study. However, no association was found between IgG_1 and blood toxic equivalent levels when these results were pooled with a second group of workers predominantly exposed in a metal recycling plant to penta- and hexachlorinated dibenzofurans, as well as to heavy metals, such as lead and cadmium (trial I + II). Halperin et al. (1998) showed lower mean IgG levels in 259 male subjects who reported exposure to TCDD during the manufacturing of 2,4,5-trichlorophenate as compared to neighborhood referents. Serum TCDD was measured in all exposed workers and in only 79 of the 243 control subjects (TCDD levels were < 20 ppt in this subsample of control subjects). When TCDD serum levels were considered, IgG did not exhibit any consistent trend in a multivariable model including age, smoking status, and alcohol consumption. Two additional small studies, which did not include IgG measurement, found a decrease in total γ-globulin (Jansing and Korff 1994) and serum IgM (Løvik et al. 1996).

In Seveso, an earlier study conducted on 44 children (3–7 years of age, 20 of whom had chloracne) shortly after the accident exhibited no change in immunoglobulin concentrations (Pocchiari et al. 1979). Other reports showed no alterations in immunoglobulin levels (Hoffman et al. 1986; Jennings et al. 1988; Jung et al. 1998; Marth et al. 1995; Michalek et al. 1999; Nagayama et al. 2001). A slight positive increase in IgG levels was found in two studies (Ott et al. 1994; Webb et al. 1989). However, these studies did not exclude subjects with concurrent medical conditions likely to increase circulating IgG levels (Ravel 1995). In one of the two investigations, the authors reported the presence of a subject affected by liver cancer who had the highest concentration of TCDD and very high IgG (Ott et al. 1994). This subject possibly acted as an outlier in the regression analysis. In the same study, another subject had liver cirrhosis. In the second investigation (Webb et al. 1989), 1 of the 12 subjects with high TCDD levels had hepatomegaly.

As for the complement components, one investigation showed a positive association between blood TCDD and C4 (Ott et al. 1994). Increases in C3 (Halperin et al. 1998) and in CH50 (Sirchia 1982) have been also reported elsewhere. Similarly, we found a weak positive association between C4 and TCDD plasma levels, but the association was not significant after adjusting for age and for other potential confounding factors.

Differences in subject characteristics, time frame of the studies, as well as in levels, nature, modality, and assessment of the exposure, may account for the conflicting results (Table 1) and certainly limit between-studies comparisons, also hindered by the dissimilar and, in some instances, incomplete presentations of the results. Our study was conducted on subjects of both sexes and within a wide age range, who had been acutely exposed to TCDD. In contrast, most other studies, including the largest investigation (Michalek et al. 1999) with 894 U.S. Vietnam veterans exposed to Agent Orange and 1,167 unexposed individuals, which showed no change in immunoglobulin levels, comprised mostly males, with long-term exposure often to multiple chemical agents, in occupational (or military) settings. Due to the type of selection, most subjects were likely young and/or healthy, and thus not representative of the general population (Li and Sung 1999). In the great majority of the investigations, the small number of subjects limited the power to detect alterations of the immunologic parameters.

In earlier studies, the measurement of the internal dose of TCDD and of other dioxin-like compounds was estimated by using surrogate measures, such as residence or presence in the contaminated facilities. Kerkvliet (1995) speculated that subjects with little or no exposure to TCDD might have also been included in these studies, accounting for the lack of significant findings. In our study, results based on TCDD plasma levels did not differ from the results based on subjects' residence in the exposed areas, but the measurement of individual plasma TCDD levels allowed for a more refined dose–response assessment.

Recently, Neubert et al. (2000) indicated various factors that can influence immunologic variables and suggested that conclusions on possible effects of exogenous agents may be drawn only when all known confounders are taken into account. The authors provided a list of possible confounders that included age, body mass, smoking, alcohol consumption, acute and chronic diseases, and use of medications. We controlled for all these factors in our analyses, in contrast to many investigations listed in Table 2.

The present study was based on a relatively small random sample of the resident population of the territory contaminated with TCDD after the Seveso accident. A similar number of subjects was drawn from the population of the surrounding noncontaminated area, which shares the same environmental, social, educational, and occupational characteristics. A detailed assessment of personal medical history and medication use was performed and compared with the results of a selection of standard laboratory tests.

The clinical significance of our finding is uncertain. The protective capacity of circulating IgG antibodies against microbial challenge is well documented (Heinzel 2000). Children and adult subjects with B-cell-related primary immunodeficiency syndromes, which exhibit reduced circulating immunoglobulins, are prone to recurrent infections and allergic diseases (Heinzel 2000). These subjects are at very high risk for non-Hodgkin lymphoma (Ziegler 1994) and increased occurrence of non-Hodgkin lymphoma has been also reported in TCDD-exposed subjects (IARC 1997). However, IgG levels found in our study sample are far above those typically observed in patients with antibody immunodeficiency disorders (≤ 350 mg/dL) (Heinzel 2000) and only one study subject had plasma IgG levels below the lower reference value (< 878 mg/dL) adopted by the clinical laboratory of the hospital.

Our finding may reflect a broader alteration of the immune system that more sensitive

markers could reveal. Lymphocyte phenotypic subsets, proliferative responses to mitogens, and basal and stimulated *in vitro* cytokine production (Colosio et al. 1999) could be assessed in future studies. In addition, genetic aberrations associated with lymphohemopoietic neoplasms (Bäsecke et al. 2002) could be tested. The results of such studies might have broad implications for public health, as current background levels of human exposure to dioxin-like compounds are close to the recently established tolerable daily intake (WHO-ECEH 2000) and thus might be sufficient to elicit toxic effects on the immune system.

In conclusion, our study showed a strong inverse relationship between plasma IgG and increasing TCDD levels. Plasma TCDD was not correlated with IgM, IgA, C3, or C4 levels. The possible long-term immunologic effects of TCDD, coupled with the increased incidence of lymphatic tumors in the area of the accident (Bertazzi et al. 2001) and the conflicting results in the literature warrant further investigation.

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